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Short communication

Biosequestration of carbon dioxide, biomass, calorific value and biodiesel precursors production using a novel flask culture photobioreactor



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ABSTRACT

Renewable, carbon neutral, economically viable alternative fuels are urgently needed to turn away the consequences of climate change. Photosynthetic capability of microalgae with respect to CO₂ fixation at various CO₂ partial pressures generated by CO₂ generating buffer (KHCO₃/K₂CO₃), increases in biodiesel precursors using IR-CO₂ sensor and modern LED lights-based modern two tier flask photobioreactor has been studied. *Chlorella vulgaris* and *Scenedesmus obliquus* were found to produce 37.11% and 32.23% of palmitic acid (C16:0) and 30.88% and 39.73% of octadecenoic acid (C18:1) of total fatty acid methyl esters (FAMES) respectively at 7.5% of CO₂ partial pressure, under the optimal values for growth. Carbon dioxide fixation rate, efficiency of conversion of biomass to calorific value and biodiesel precursors were also estimated.

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1. Introduction

Greenhouse gases are responsible for global warming and their major source is fossil fuel. Renewable, carbon neutral, economically viable alternatives to fossil fuels are urgently needed to turn away the impending oil crisis and the

spectacular consequences of climate change. Many reports have suggested using flue gas as carbon origin for microalgal cultivation, which could combine biofuel production with current CO₂ mitigation strategies [1–3]. Today, the potential value of microbial, and particularly microalgal photosynthesis to produce biofuel is widely recognized [4,5]. However, the influence of high CO₂ concentration on microalgal growth must

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be investigated [6,7]. Recently, biodiesel fuel has received much attention since it is made from non-toxic, biodegradable, and renewable resources, and its use leads to a decrease in the emission of harmful air pollutants [8], but it will be necessary for the microalgae to have a high calorific value if they are to be used as a biodiesel. The main contribution to the calorific value of the cells is from their carbohydrates, protein and lipid [9,10]. United Nations have imposed carbon credit since 2010 and its estimated carbon prices are reaching up to US \$ 270/tonne [10]. Among various strategies for mitigating CO₂, the biological sequestration of CO₂ using photosynthetic microalgae has receiving considerable attention, as microalgae have a high CO₂ fixation capability and produce value added products through their biomass. A variety of microalgae species have been applied in CO₂ fixation, including *Chlorella vulgaris*, *Scenedesmus obliquus*, *Chlamydomonas reinhardtii*, *Spirulina* sp. and *Botryococcus braunii* [11–13].

The aim of this research was to screen potential microalgae for high carbon dioxide fixation along with disparity in calorific value at a particular partial pressure of CO₂ in a flask culture photobioreactor. The carbon dioxide fixation rate, calorific value and biodiesel precursor of microalgae were also evaluated under different CO₂ partial pressure with a novel IR-CO₂ sensor in LED-light based flask culture photobioreactor experiments.

2. Methods

2.1. Microorganism and culture media

The microalgal cells used in this study were *Chlorella* sp., obtained from the Indian Agricultural Research Institute (IARI), New Delhi, India [6] and *Scenedesmus obliquus*, *Chroococcus* sp and *Chlamydomonas* sp. that were obtained from Environmental Health Division, CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), Nagpur, India. Microalgal strains cultured in the medium BG-11 [14] and incubated at $28 \pm 1^\circ$ 86 C at light intensity of $320 \mu\text{mol m}^{-2} \text{s}^{-1}$ with 16 h light, 8 h darkness (16:8). Flask culture photobioreactor used for cultivation was shaken manually. The microalgae were cultivated for 3 weeks in an enclosed experimental CO₂ set up to investigate their growth characteristics under various CO₂ partial pressures in flask culture photobioreactor.

2.2. Experimental set-up for microalgae growth under various CO₂ partial pressures

The microalgal cultures as mentioned above were incubated in culture flask photobioreactor made up of a 250 mL Erlenmeyer flask (Top) and a round bottom flask (Bottom). The set up is shown in Fig. 1 with the upper flask for microalgal growth and the lower flask contains 100 mL of CO₂ generating buffer mixture (KHCO₃/K₂CO₃) and buffer was replenished after every 48 h to keep CO₂ partial pressure constant. The accurate CO₂ partial pressure in headspace of the flask was optimized to 1.4%, 3.0%, and 7.5% by using different concentration of buffer mixture and monitored by infra-red CO₂ sensors along with one flask kept as control without buffer. The flask system's working volume of 100 mL was kept air

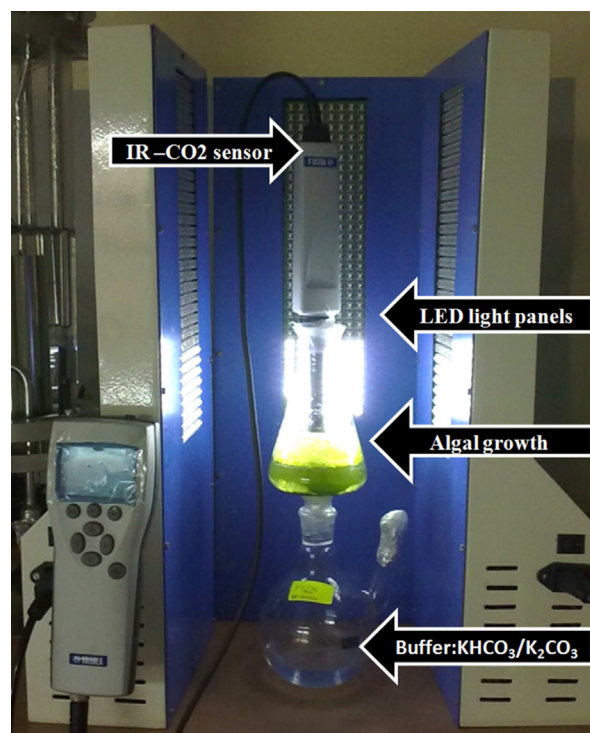


Fig. 1 – Experimental set up showing culture flask photobioreactor with IR-CO₂ sensor at the top of upper flask, artificial CO₂ generating buffer (KHCO₃/K₂CO₃) in the bottom flask and LED based light panels.

tight by using parafilm. Light intensities, temperature and CO₂ concentrations were kept constant in accordance with the above mentioned microalgal growth conditions.

2.3. Determination of dry cell weight

The dry cell weight was measured by filtering aliquots of known cell numbers on pre-weighed GF/C filter paper (Whatman, UK). The filtered cells were dried at 80 °C for 24 h.

2.4. Light intensity

Light intensity was provided by three Light Emitting Diodes (LED) panels and determined with light meter (LX-107HA, USA). This light meter gives a reading in Klux that can be converted to $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the measured light intensity ($1 \text{ Klux} = 19.5 \mu\text{mol m}^{-2} \text{s}^{-1}$).

2.5. Carbon dioxide (CO₂) determination

Carbon dioxide concentration was analyzed by iBRID MX6 sensor (Industrial Scientific, UK) and CO₂ partial pressure in experimental set up was also cross checked by an Infra-Red CO₂ sensor MI 70 (Vaisala, Finland).

2.6. Determination of calorific values (CV)

The calorific values of dried algal cells were determined using a Digital bomb calorimeter (Optical Technology, Delhi,

India). Microalgal cells were harvested from BG-11 media. The algal biomass samples (100 mL) were filtered onto cellulose nitrate filters (Whatman No.1) and dried at 60 °C for about 4 h. The biomass of the sample was combusted in the calorimeter and benzoic acid was used as a calorific standard. Calorific value (CV) of biomass was calculated as indicated in Equation (1).

$$\text{Calorific value} = \frac{2325 \times (\text{Final temp} - \text{Initial temp}) - 45}{\text{Total biomass weight}} \text{ kcal/g} \quad (1)$$

with 2325 (kcal) being water equivalent energy.

45 is CV of Nichrome wire & cotton thread (kcal)
Final & Initial Temperature (°C) & (1 kcal = 4.1868 kJ)

2.7. Determination of carbon content in biomass

The carbon content of the dried samples was determined using a model Vario EL III CHNS analyzer (Elemental Analyser System GmbH, Germany) and calibrated using a Perkin Elmer sulphanic acid standard to represent 100% carbon. CO₂ fixation by each organism was calculated from the CHNS biomass carbon content values.

2.8. Calculation of CO₂ fixation (g CO₂ L⁻¹ d⁻¹)

$$\text{CO}_2 \text{ fixation rate} = 0.50P \times 44 \times 12^{-1} \quad (2)$$

where: P – Biomass productivity
44 – Molecular weight of carbon dioxide (g)
12 – Atomic weight of carbon

CO₂ fixation rates were, generally calculated from the biomass productivity by using 50% as the carbon content of dried cells [15]. But in the present study CO₂ fixation rates were calculated from values carbon content, determined by the CHNS analyser.

2.9. Extraction and determination of biodiesel precursors from microalgal biomass

Extraction of lipid was done following the protocol of Bligh and Dyer [16]. The total lipids were measured as fatty acid methyl esters (FAME) following the direct transesterification method using a gas chromatograph (GC) equipped with a FID detector (Perkin Elmer, USA). Analysis was done using a SP-2560 column (100 m × 0.25 mm ID, 0.20 μm) supplied by Sigma (Germany). 5–8 μl of sample was injected into the GC. The GC conditions were: injector temp: 260 °C; column: 140 °C and detector temp: 260 °C. Helium was used as carrier gas with a flow rate of 1 mL/min [1,6].

2.10. Statistical analysis

Each measurement was done in Triplicate. The means and standard deviations of the experimental results were calculated using Minitab 15 English software and MS-excel.

3. Results and discussion

3.1. Partial pressure of CO₂ in K₂CO₃/KHCO₃ buffer mixture

Carbon dioxide is the carbon source employed for photosynthesis and depending on various algal species. CO₂ can

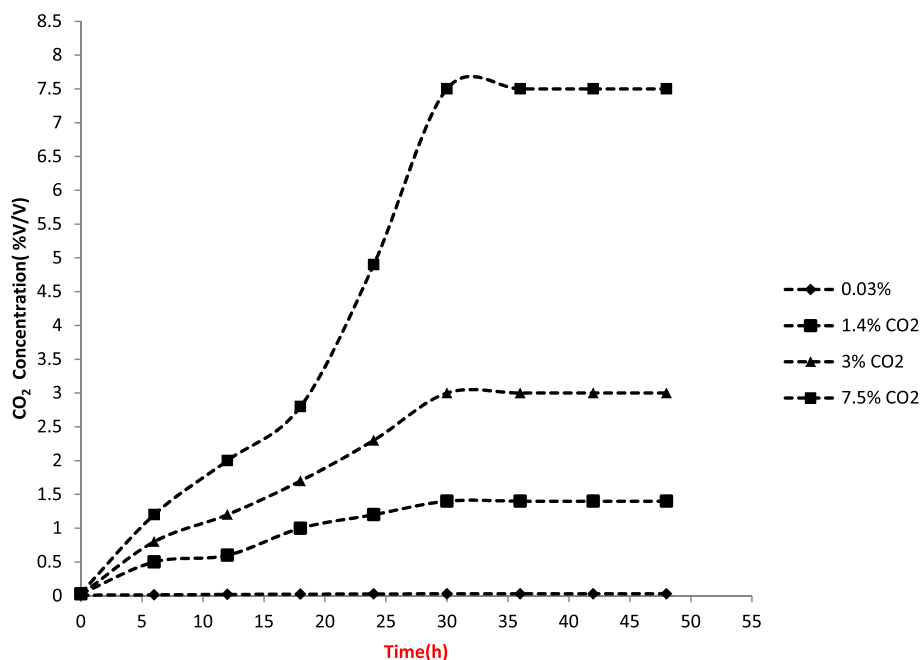


Fig. 2 – Various CO₂ partial pressures (% v/v) generated by CO₂ generating buffer (KHCO₃/K₂CO₃), data monitored by IR CO₂ sensor up to 48 h (at 6 h interval).

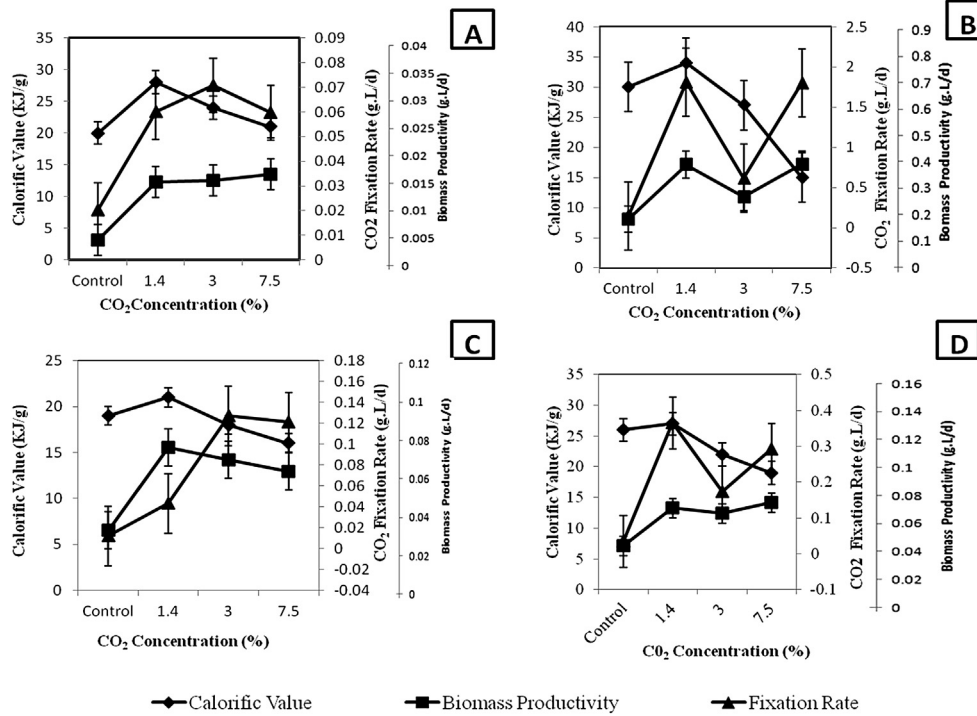


Fig. 3 – Effect of CO₂ partial pressure (%v/v) generated by KHCO₃/K₂CO₃ buffer mixture on biomass productivity (g°L⁻¹ d⁻¹), CO₂ fixation rate (g L⁻¹ d⁻¹) and calorific value (kJ/g) A) *Chlorella* sp., B) *Scenedesmus obliquus*, C) *Chroococcus* sp. and D) *Chlamydomonas* sp.

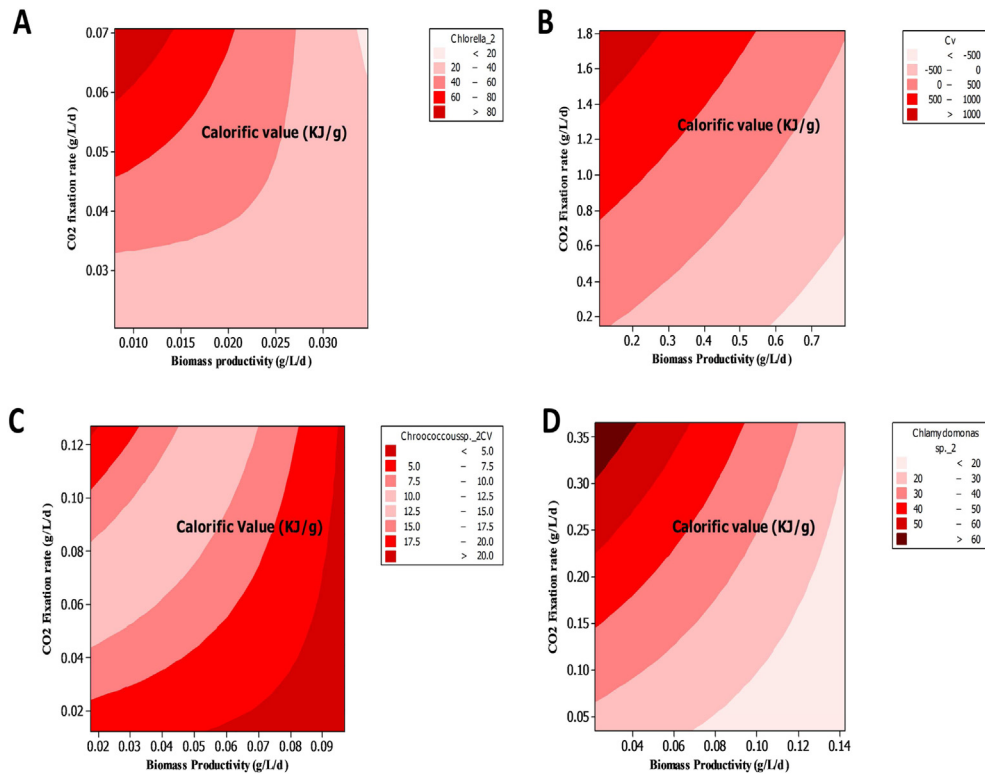
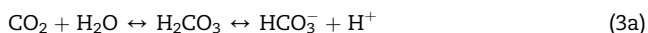


Fig. 4 – Counter plot of the flask photobioreactor calorific value vs. biomass productivity and CO₂ fixation rate at 7.5% CO₂ (A) *Chlorella* sp., (B) *Scenedesmus obliquus*, (C) *Chroococcus* sp. and (D) *Chlamydomonas* sp.

be absorbed in dissociated form such as HCO_3^- and CO_3^{2-} [17].



Carbon dioxide, dissolved in water is partially hydrated to carbonic acid (a reaction that is greatly accelerated by the enzyme, carbonic anhydrase). Carbonic acid is in equilibrium with bicarbonate and there is an apparent $\text{pK}_a = 6.10$ for the reaction (3a). The backward reaction of Equation (3a) is also true. When a carbonate salt is dissolved in water, there is a partial pressure of CO_2 on its surface. According to Le Chatelier's principle, if any of the reactants are removed, then the equilibrium shifts towards left and the backward reaction is used. In present flask photobioreactor system, the CO_2 is utilized by the algae continuously, thus favoring the backward reaction of Equation (3a) resulting in production of more CO_2 . This reaction continues until all the HCO_3^- is used up. But the HCO_3^- ions are replenished by reaction (3b). On the whole, a continuous emission of CO_2 occurs until CO_3^{2-} ions are used up. A catalyst speeds up the rate of a reaction by providing additional mechanism(s). Since no catalyst is added in this case, both the reactions are slow. Thus, the CO_2 mass transfer is achieved slowly and steadily in the present designed flask at various CO_2 partial pressures as shown in Fig. 2. Lee and Palsson further investigated LED-based photobioreactors (PBR) for high-density algal biomass [17].

3.2. Characteristics of algal biomass at different CO_2 concentration

This photobioreactor system enables us to discover several microalgal strains among the studied naturally isolated species and the obtained results show the difference in the biomass productivity, carbon dioxide fixation rate and calorific value of four microalgal species. All the species used in this study were confirmed to grow upto 7.5% CO_2 partial pressure with difference in biomass productivity. Among the four species studied, *Scenedesmus obliquus* showed maximum productivity $0.7916 \pm 0.07 \text{ g L}^{-1} \text{ d}^{-1}$ as shown in Fig 3. The increase in productivity always depends on the photosynthetic efficiency of microalgae strains, photobioreactor design, improve temperature tolerance, biomass growth rate and increase biomass yield under the influence of light [4]. Hence the current novel flask culture photobioreactor study confirmed the other studies in the literature. The CO_2 fixation rate and calorific value were found to be $1.813 \pm 0.32 \text{ g L}^{-1} \text{ d}^{-1}$ and $34 \pm 0.1 \text{ kJ/g}$ at 1.4% CO_2 . In a previous study, Yoo et al. [2] reported $217.50 \pm 11.24 \text{ mg L}^{-1} \text{ d}^{-1}$ at 10% CO_2 . Morais and Costa [12] reported that maximum biomass productivity for *Scenedesmus obliquus* was $0.14 \text{ g L}^{-1} \text{ d}^{-1}$. Thus the present study confirmed that biomass productivity for *Scenedesmus obliquus* was 3–4 times higher than those of previous studies and all microalgae could be grown in the atmosphere of 0.03, 1.4, 3, and 7.5% CO_2 partial pressure in the flasks culture photobioreactor. Fig. 4 shows counter plot of the flask photobioreactor calorific value vs. biomass productivity, the

Table 1 – Fatty acids composition of *Chlorella vulgaris* and *Scenedesmus obliquus* at 3% CO_2 .

| Fatty esters (%) | Myristic (C14:0) | Palmitoleic (C16:1) | Palmitic (C16:0) | Linoleic (C18:2) | Linolenic (C18:3) | Octadecenoic (C18:1) | Arachidic (C20:0) | Erucic (C22:1) | Saturated Fatty Acids (SFAs %) | Monounsaturated Fatty Acids (MUFAs %) | Polyunsaturated Fatty Acids (PUFAs %) | Total (%) |
|-----------------------------|------------------|---------------------|------------------|------------------|-------------------|----------------------|-------------------|----------------|--------------------------------|---------------------------------------|---------------------------------------|-----------|
| <i>Chlorella vulgaris</i> | 0.05 | 5.60 | 35.08 | 07.34 | 20.44 | 30.81 | 0.25 | ND | 35.38 | 36.41 | 27.78 | 99.57 |
| <i>Scenedesmus obliquus</i> | 0.70 | 2.59 | 32.23 | 14.63 | 9.89 | 39.73 | 0.19 | ND | 33.12 | 42.32 | 24.52 | 99.96 |

ND: Not Detected.

Table 2 – CO₂ fixation rates of four algae species grown at different CO₂ partial pressure.

| CO ₂ partial pressure (%) | CO ₂ fixation rate (g L ⁻¹ d ⁻¹) | | | | Reference and remark |
|--------------------------------------|--|------------------------|--------------------------|---------------|---|
| | <i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> | <i>Chroococcus</i> sp. | <i>Chlamydomonas</i> sp. | | |
| Control | 0.0203 ± 0.17 | 0.1458 ± 0.21 | 0.0121 ± 0.22 | 0.0347 ± 0.27 | Present study (Novel flask culture photobioreactor) |
| 1.4 | 0.06 ± 0.23 | 1.813 ± 0.32 | 0.0434 ± 0.27 | 0.3654 ± 0.35 | |
| 3 | 0.0708 ± 0.31 | 0.6188 ± 0.43 | 0.127 ± 0.35 | 0.1732 ± 0.30 | |
| 7.5 | 0.0597 ± 0.44 | 1.799 ± 0.33 | 0.121 ± 0.37 | 0.292 ± 0.32 | |
| 2 | 0.43 | – | – | – | |
| 10 | – | 0.29 | – | – | [22] (Erlenmeyer flask) |
| 30 | – | – | – | NA | [23] (Batch reactor) |

Abbreviation: NA = Not Available.
Note: Each data indicates the mean ± SD which were measured from three independent culture.

biomass productivity for *Chroococcus* sp. was found to be 0.0969 ± 0.09 g L⁻¹ d⁻¹ at 1.4% CO₂ partial pressure in these flasks photobioreactor and that of for *Chlamydomonas* sp. was found to be 0.1425 ± 0.12 g L⁻¹ d⁻¹ at 7.5% CO₂ partial pressure. *Chlorella* sp. has also shown maximum biomass productivity 0.0347 ± 0.05 g L⁻¹ d⁻¹ at same partial pressure.

3.3. Biodiesel precursors

At 7.5% of CO₂ partial pressure *Chlorella vulgaris* and *Scenedesmus obliquus* showed 36.11% and 32.23% of palmitic acid (C16:0) and 30.88% and 39.73% of octadecenoic acid (C18:1) of total fatty acid methyl esters (FAMES) from *Chlorella vulgaris* and *Scenedesmus obliquus*, with respect to the optimal values for growth, respectively. This result concurs with that in Table 1. The fatty acid composition in green algae has been reported to range from C-14:0 to C-20:0 [18] and majority of lipid-producing algal species have a similar lipid profile which is, in general, equivalent to vegetable oil from land plants suitable for biodiesel production [18]. The concentration of values of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of *Chlorella vulgaris* and *Scenedesmus obliquus* (Table 1) found to be (SFA + MUFA + PUFA) of 99.57% and 99.96%, respectively. However, values obtained for the concentrations of saturated, monounsaturated and polyunsaturated fatty acids showed that *Chlorella vulgaris* and *Scenedesmus obliquus* were predominant in saturated and mono-unsaturated fatty acids (Table 1).

These results reveal that the production of biodiesel from microalgae oil will indeed prove profitable and when grown under appropriate nutrient and CO₂ conditions would lead to ideal high-biodiesel precursors yielding process and efficient CO₂ sequestration for primary screening.

3.4. CO₂ consumption rate and calorific value of algal biomass

The current study compared the carbon dioxide fixation rate and carbon dioxide consumption rates of four microalgae species at various carbon dioxide atmospheres in the flask culture photobioreactor. However, the CO₂ fixation efficiency would differ with input feeding factors, such as carbon dioxide rate, media and light intensity capable of influencing the photosynthesis efficacy, including carbon dioxide

amalgamation ability, carbon dioxide retention and shading effects of microalgae [19,20].

Moreover, the carbon dioxide removal efficacy can be evaluated by carbon dioxide consumption rate calculated as shown in Eq. (2). The cultures used for CO₂ fixation and sequestration namely *Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlamydomonas* sp and *Chroococcus* sp showed a CO₂ fixation rate of 1.81 g L⁻¹ d⁻¹, 0.36 g L⁻¹ d⁻¹, 0.04 g L⁻¹ d⁻¹ and 0.06 g L⁻¹ d⁻¹ respectively at 1.4% CO₂ partial pressure (Table 2). Comparative CO₂ fixation rate of same microalgae strains also tabulated in Table 1 [21–23]. Thus, photosynthetic capability of microalgae with respect to various CO₂ partial pressure could be demonstrated. The cultures used for CO₂ fixation and sequestration namely *Scenedesmus* sp, *Chlorella vulgaris*, *Chlamydomonas* sp and *Chroococcus* sp had a calorific value of 34 kJ/g, 28 kJ/g, 27 kJ/g and 21 kJ/g respectively at 1.4% CO₂. High calorific value content and high lipid content were consistently reported in the literature. The calorific values of these strains are higher than some of the values reported in the literature, suggesting the potential of these isolates to be used in various applications. Hence present research could be helpful in rapid screening of algal species which can sequester higher percent of CO₂ and provide biodiesel precursors and high calorific value.

4. Conclusion

The application of microalgae for CO₂ sequestration and high caloric value are key to the commercial success of carbon capture and microalgae-based biofuel. Hence, the experiments carried out to screen the different algal species under various partial pressures of CO₂ in novel flask photobioreactor which showed photosynthetic CO₂ fixation was strongly reliant on the CO₂ concentration supplied during growth of algae. Hence, the flask culture photobioreactor study carried out might be useful for out for screening of microalgal strain, to find out CO₂ sequestration capability, calorific value and biodiesel precursors.

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